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Implementation of the clinical practice of liquid biopsies for thoracic oncology the experience of the RespirERA university hospital institute (Nice, France)

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ABSTRACT

According to international guidelines, it is mandatory to evaluate predictive biomarkers of targeted therapies and the response to immune check point inhibitors for patients with non-squamous non-small cell lung cancer (NS-NSCLC). For this purpose, a tissue sample is nowadays the gold standard, but biofluids, particularly peripheral blood, can be a complementary and sometimes an alternative approach to assess the status of different druggable genomic alterations of advanced NS-NSCLC. A liquid biopsy (LB) is an attractive approah for better treatment decision-making by thoracic oncologists for NSCLC patients in daily practice at both initial diagnosis and tumor progression. We describe the experience of a clinical and molecular pathology laboratory (LPCE, Nice, France) developing the use of in-house LB in thoracic oncology. Moreover, we report the changes in clinical care, the advantages, but also the possible constraints associated with implantation of LB in routine clinical practice.

Introduction

Tissue samples from both biopsies and surgically resected specimens are the gold standard not only for diagnosis and histological subtype characterization of non-small cell lung cancers (NSCLC), but also for evaluation of the expression of PD-L1 and of druggable genomic alterations [1,2]. However, tissue sampling can present a number of limitations, notably since it is an invasive and hardly repeatable approach, which can be associated with negative results when an insufficient amount of material is obtained for molecular testing. In this regard, liquid biopsies (LBs) hold many advantages, and can participate in the evaluation and monitoring of the response to treatment [3]. Despite its potential, different issues still hamper adoption of LBs in daily practice.

The objective of this article is to evaluate the usefulness but also the current challenges associated with developing in-house LB molecular testing for routine clinical practice for NSCLC. After a brief landscape view of the current and possible future predictive biomarkers of NSCLC, we provide practical insight into the implementation in thoracic oncology of LBs based on the experience developed in a clinical and molecular pathology laboratory [Laboratory of Clinical and Experimental Pathology (LPCE), Nice, France]. We then identify some of the constraints and limitations of using LBs in daily practice. Finally, some perspectives for LB in the field of thoracic oncology for better care of lung cancer patients will be developed.

Current and future predictive biomarkers to identify in non-small cell lung cancer

According to international guidelines, it is nowadays recommended to look for *EGFR*, *KRAS*, *BRAF*, *HER2*, *MET* exon 14 mutations and *ALK*, *ROS1*, *RET*, *NTRK* rearrangements, as well as the PD-L1 expression in tumor cells, before initiating first-line treatment of stage IIIB/IV non-squamous non-small cell lung carcinomas (NS-NSCLC) [4]. Moreover, following the recent published results of the ADAURA study, it is now recommended to look for *EGFR* mutations and *ALK* rearrangements in stage IB-IIIA NS-NSCLC [5]. It is more and more admitted that all these genomic alterations have to be evaluated using next generation sequencing (NGS) approaches [4]. All these biomarkers, except PD-L1, can be evaluated not only on tissue samples but also in different fluids (blood, cerebrospinal fluid, pleural or pericardial effusion).

Due to the development of clinical trials associated with different targeted therapies or with immune or immune-chemotherapy, it may become mandatory to look for additional molecular alterations in the future in routine clinical practice. Therefore, according to the ESMO scale

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for clinical actionability of molecular targets (ESCAT), the status of other genes, notably *NRG1, BRAC1/2, PI3KA* should be evaluated in the near future before administration of some new targeted therapies for NS-NSCLC [4]. In addition, mutations present on other genes such as *STK11*, *KEAP1*, but also *SMARCA4* or *NOTCH*, can have an impact on the efficacy of immunotherapy and should soon provide better care of NS-NSCLC patients [6–9]. It may also be of interest to integrate in the future the detection of microsatellite satellite instability (MSI), although very rare in NS-NSCLC, and of different genomic alterations in squamous cell NSCLC including the *FGFR* genomic alterations [10,11]. Finally, a number of studies have shown controversial results regarding the tumor mutation burden and, at least in Europe, it is not currently recommended to evaluate this biomarker of response to immunotherapy in daily practice, but may be of interest in the future [12]. We have to keep in mind the interest of analyzing the genomic landscape of the tumor for each druggable mutation, while integrating the different associated co-mutations. Therefore, these co-mutations, such as *TP53* mutations associated with *EGFR* mutations, can have an impact on the response to tyrosine kinase inhibitors (TKIs) and the overall survival of patients with NSCLC [13].

Implementation of liquid biopsies in the routine clinical practice of a single institution

In 2014 we decided to perform LBs for certain patients because they could not get access to target therapies (at that time TKIs targeting *EGFR* mutations) subsequent to failed molecular tests from tissue samples or lack of access to tests. This was the case when no tissue was left over after histological and immunohistochemical evaluation, if a low level and/or quality of extracted nucleic acids was available, or if a tissue biopsy was not performed. On rare occasions a LB was performed for a patient who needed urgent treatment since the turnaround times to obtain results from a tissue biopsy can be rather long [14]. We were able to set up LBs in our laboratory since the French Ministry of Health reimbursed molecular testing in oncology independently of the nature (tissue or biofluid) of the sample [15].

Initial requests to set up liquid biopsies at the LPCE

The use of TKIs targeting the main druggable *EGFR* mutations (exon 19 deletions and p.L858R mutation) was associated with the possibility of detecting these two genomic alterations in the cf-DNA of advanced NSCLC patients [16,17]. Therefore, these *EGFR* mutations could be detected with cf-DNA before initiating first or second generation TKIs, notably when no tissue sample was available or in the absence of extracted nucleic acid from tumor samples. In this context, we set up one gene sequencing molecular testing using qPCR [16,17]. Moreover, since the main mechanism of resistance that develops in patients receiving these first and second generation TKIs was the *EGFR* p.T790 M mutation, which is easily detected with cf-DNA, it was of interest to first perform a one sequencing molecular test with a blood sample, notably to avoid a renewed invasive tissue biopsy at tumor progression [18,19]. This blood test was regularly performed in the daily practice of our laboratory from 2010 up to 2020 (Fig. 1). The development of TKIs targeting *ALK* rearrangement led us to identify in parallel *ALK* rearrangements at baseline with cf-DNA when no tissue sample was available [20]. Subsequently, we received an increased number of blood samples from the clinical department of our institution and from different hospitals and private clinics in the south of France.

Increasing the number of liquid biopsies at the LPCE

An increase in the demand and thus practice of LB was associated with the possibility for the physician to be informed of genomic alterations accessible to TKIs and to then make decisions concerning treatment when no tissue sample was available. In addition, many requests for LBs were made at tumor progression due to the sensitive and non-

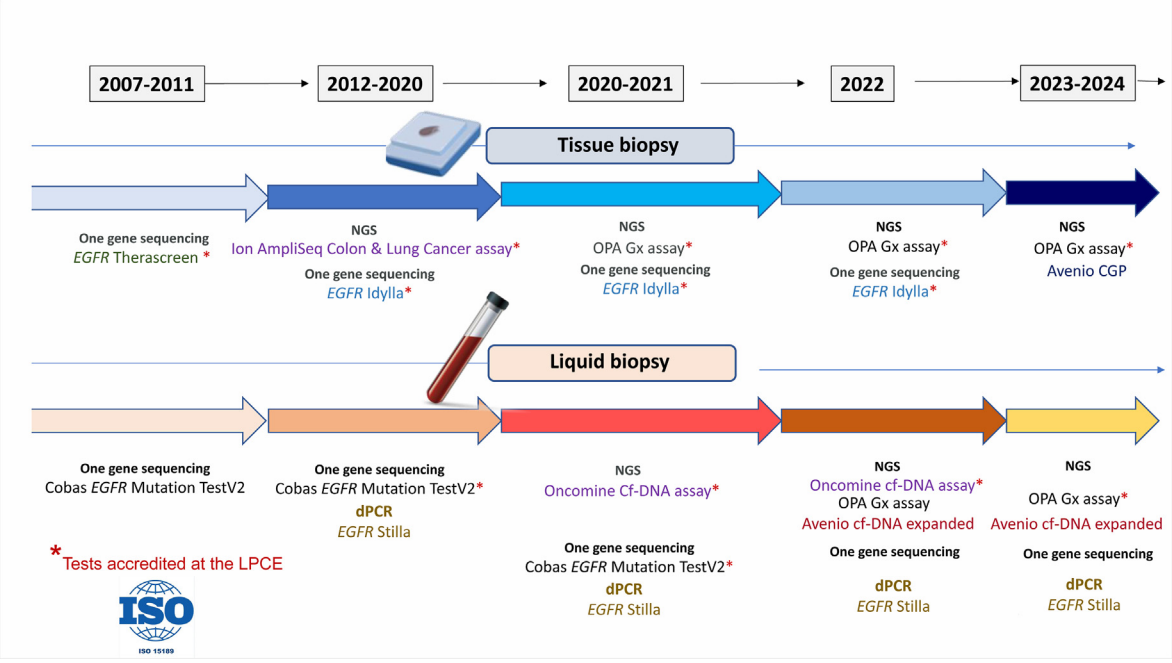

Fig. 1. Molecular biology testing from tissue and liquid biopsies at the laboratory of clinical and experimental pathology laboratory from 2007 to 2023. Different one gene sequencing, digital PCR (dPCR) and next generation sequencing (NGS) assays have been use according the sample types and the time period: Therascreen EGFR RGQ PCR Kit (Qiagen, Hilden, Germany); Idylla TM EGFR Mutation Test (Biocartis, Munchen, Belgium); cobas® EGFR Mutation Test v2 (Roche Diagnostics, Basel, Switzerland); Oncomine™ Lung cfDNA Assay (Thermo-Fisher Scientific, Waltham, MA, US), Ion AmpliSeq Colon and Lung Cancer Research Panel v2 (Thermo-Fisher Scientific), Oncomine™ Precision Assay GX (Thermo-Fisher Scientific). 3-Color Crystal Digital PCR™ assays for EGFR mutation (Stilla, Paris, France). AVENIO ctDNA Expanded Kit (Roche Diagnostics, Basel, Switzerland), AVENIO Tumor Tissue CGP Kit (Roche Diagnostics). Tests with an asterik correspond to tests accredited according to the ISO 15189 standard. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

invasive nature of the test for the detection in around 50% of cases of the *EGFR* p.T790 M mutation [19]. The detection of different mutations was associated with the possibility of proposing alternative therapeutics in second-line. So, in this situation the oncologists were aware that sometimes a second biopsy was not necessary in these patients, thereby avoiding hospitalization, a new endoscopy with bronchial biopsies and/or a CT scan with transthoracic biopsies, which are much more invasive, time consuming and less cost effective than LBs [21,22]. In parallel, the development of mechanisms of resistances in patients treated with first and second generation ALK inhibitors could be detected with cf-DNA at tumor progression, mainly the different *ALK* mutations [23]. We first detected at baseline *ALK* rearrangements using single gene molecular testing with blood samples [20]. The recognition of new molecular targets and mechanisms of resistance led us to develop NGS technologies with LBs [24] (Fig. 1).

Liquid biopsies in the context of new targeted therapies in thoracic oncology

The adoption of third generation TKIs targeting active *EGFR* mutations has revolutionized the treatment and care of patients with advanced NSCLC [25]. Therefore, most thoracic oncologists in European countries now use third generation TKIs in first-line for *EGFR* mutated advanced NSCLC. Despite an initial tumor response, the development of mechanisms of resistance lead to renewed biopsies at tumor progression. The main mechanism of resistance is the *MET* amplification, which occurs in 20% of cases, while other genomic alterations are less frequent [26]. In contrast to the *EGFR* T790 M mutation that can be easily detected with cf-DNA, the assessment of *MET* amplifications is easier with tissue than with a LB. Therefore, the practice of LBs at tumor progression has decreased slightly for patients receiving osimertinib for the benefit of renewed tissue biopsies. Similarly, the introduction of a new generation of ALK inhibitors (such as alectinib) seems to have limited a little bit the practice of LBs at tumor progression. The progressive development of therapies targeting different genomic alterations present on other genes (*BRAF, KRAS, MET, HER2, NTRK, RET, ROS1)* led us to use NGS not only with tissue samples but also with blood samples [24].

In-house liquid biopsies use

Thanks to constant dialogue between the oncologists, the radiologists, the surgical and molecular pathologists, different algorithms have been set up to develop LBs in thoracic oncology (Fig. 2). Initially LBs were reserved for certain patients, but since 2022 we have performed concurrently tissue and LB testing for all patients diagnosed or suspected radiologically to have an advanced lung cancer [27,28]. As described above, to test the use of LBs we first performed a single gene assay for *EGFR* and assessment of the *ALK* status [6]. Then, we used in-house NGS with small and medium sized panels to test LBs. This strategy was appropriate since in-house testing allowed us to decrease the turnaround time to obtain the results compared to outsource testing in commercially available platforms outside France. Moreover, the cost and level of reimbursement were more effective when using molecular biology platforms in our hospital. In addition, it was easier in this setting to get access to the raw data, to decide rapidly whether to repeat the experiments/LBs in the case of uncertain results. We were also able to keep the leftover extracted DNA in the biobank for further research programs or controls. From our point of view the use of medium panels with LBs at the initial diagnosis or at the first progression of the tumor in thoracic oncology was adequate to take decisions concerning treatment in our daily practice.

Tests validation and external quality assurance programs for LBs use

The different in-house panels used over the years (both for tissue biopsies and LBs) as well as the corresponding accreditation notification for the different tests are show in Fig. 2. Briefly, we have to distinguish between the CE-IVD (Thermo-Fisher Scientific) and the RUO (Roche Diagnostics) panels. In this context, only molecular testing developed with CE-IVD panels were accredited according to the norm ISO 15189 at the LPCE (Fig. 1). Moreover, to maintain accreditation of this activity we participated each year in external quality assurance schemes such as the Gen&tiss LBs program in France [29].

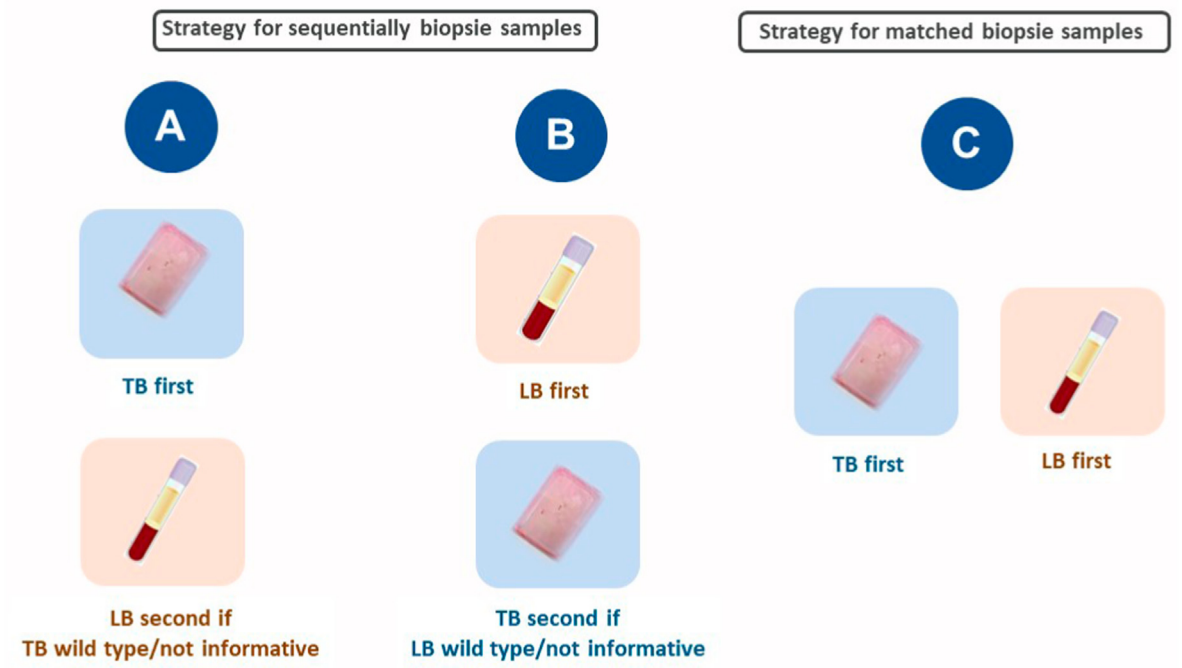

Fig. 2. Algorithm proposals for samples use for predictive biomarkers testing in thoracic oncology at the initial diagnosis of advanced non-small cell cancers. TB: tissue biopsy. LB: liquid biopsy.

Challenges and constraints of in-house implementation and use of liquid biopsies in thoracic oncology

Despite being a promising technique in thoracic oncology with several potential applications, LB still has different limitations to its widespread use in many hospitals. Therefore, some issues must be overcome for LB use in daily practice (Table 1).

Practical issues for pre-analytical processing

One important issue we experienced when LBs were initially set up in our institution concerned mastering the pre-analytical phase and the workflow of the different steps, from blood sampling to the medical report. Different parameters had to be controlled. The different steps of the pre-analytical phase were set up according to international recommendations [30]. In particular, careful attention was given to the types of buffers to be used (EDTA versus Streck BCT tubes) for the blood samples and to the logistical issues such as the time of transportation from the clinical site (own institution or external sites) to the laboratory.

Implementation of different NGS based technologies for analysis of liquid biopsies

Technologies for molecular testing with cf-DNA have evolved over the years from single gene testing to multiplex hot-spot testing and comprehensive genome profiling. NGS is now the most attractive approach for cf-DNA genomic assessment, but different panels and technologies (eg, amplicon-based sequencing *versus* hybrid capture sequencing) can be developed. According to the indication for a LB at initial diagnosis and at tumor progression and according to the different lines of treatment, the sensitivity and specificity have to be evaluated comparatively [31].

Table 1
Challenges to in-house implementation of liquid biopsies in thoracic oncology.

Pre-analytical issues
Standardization of blood collection (volume) and stabilization (type of tube: EDTA *versus* Streck BCT tubes)
Information concerning transport and traceability up to sample registration in the laboratory
Standardization of centrifugation, aliquoting, DNA isolation and storage
DNA isolation and quality/quantity assessment
Analytical issues
Selection of the technologies for analyzes with ct-DNA (PCR and/or dPCR and/or NGS)
Selection of the NGS panel (s): small (<50 genes) and/or medium (50 genes) and/or large (>300 genes) panels
Post analytical issues
Interpretation and reporting of genomic alterations (standardization of the report)
Set up a comprehensive description and interpretation of the mutational profile of the assessed biomarkers
Informatics for report transmission
Follow the international recommendation in respecting the turnaround times to submit the report
Accreditation and external quality assessment
Accreditation of tests with liquid biopsies according to the ISO 15189 norm
How to select the tests when using liquid biopsies to be accredited
Selection of recognized national of international programs for external quality assessment
Management of the laboratory
Specific training for the laboratory staff
Promote as much as possible the automation of the pre-analytical and analytical phases
Reinforce the capacity of storage genomic data
Possibility to increase the number of staff members
Interaction with the physicians
Establish continued dialogue for improvement of processing
Discuss performing a liquid biopsy
Collective interpretation of the results/report
Convince the administation and the financial bodies
Anticipate the activity, cost, reimbursement of liquid biopsy testing at the local and national level
Set up educational training
Develop master classes for academic and private partners to
- Harmonize routine clinical practices and transmission of guidelines
- Practical training on site

Impact of an increase in activity in the laboratory on the workload of staff members and on the increase in cost

Setting up LBs in our institution changed the clinical practice, increased the volume of genetic testing and consequently, the cost of healthcare. The evolution of knowledge into genomics and medical genetics has also elicited the need for accurate estimation of the impact of the cost of genetic testing on the healthcare system in relation to the clinical utility. Moreover, the analysis of the time required for basic procedures (i.e. nucleic acid extraction and quality control along with sample acceptance and codification) suggested that their centralization in expert centers, along with automation in shared work-units could help to improve efficiency and reduce the cost of personnel for these processes. Automation of laboratory procedures should have a favorable impact on the global quality of the results, as it reduces drastically the number of errors. Estimation of the workload may serve to better evaluate the effort required by the personnel to optimize the molecular workflow, moving when possible toward increased automation. These values may also have a significant impact on setting up strategies of recruitment of personnel by hospitals. So, costs are a crucial issue to be faced by the health care system and are of outmost importance today, when new NGS strategies play an increasing role and consequently have an economic impact on the medical field.

Dissemination of good practices of prescribers, oncologists and pathologists

We decided to develop educational training sessions to better inform all the actors participating in liquid biopsy analysis in thoracic oncology concerning the usefulness of the goals of ordering this test. Moreover, these sessions aimed to disseminate the good practices, notably to master the pre-analytical, analytical and post analytical phases, but also to educate the oncologists and pathologists on interpretation of the result. Part of this training was developed as masterclasses open to different external teams thanks to the support of AstraZeneca Pharmaceutical Company (Global Diagnostics), who identified our laboratory as a center of LB expertise in Europe. Moreover, our laboratory was selected by the European Society of Pathology as an advanced training center for molecular pathology with an emphasis on LB [32].

The selection of the LB assay may influence the treatment of the patient [33]. Many factors influence the clinical validity and clinical utility of LBs testing [33]. One important point is to discuss the possibility that pathologists initiate reflex testing with LBs in a similar way to initiation of reflex testing for biomarkers with tissue samples [34]. Finally, according to the international guidelines and/or daily practice the introduction of different algorithms and indications of LBs can be proposed [24,27,28,30,35–38].

Availability and accessibility of NGS in liquid biopsies in thoracic oncology in Europe

One bottleneck to using LB in thoracic oncology lies in the discrepancies in its availability and accessibility in Europe, notably in thoracic oncology [[39,40]. Thus, despite guideline recommendations, significant discrepancies in access to NGS persist across Europe, primarily due to constraints in reimbursement [41].

Perspectives

LB is going to have a leading place in thoracic oncology with a widespread adoption in routine clinical practice and LB will certainly reshape the diagnostic approach and different algorithms for NSCLC care. In this context, different perspectives should be considered now (Table 2).

Table 2
Perspectives for in-house liquid biopsies in thoracic oncology. CGP: comprehensive genomic profiling. NSCLC: non-small cell lung cancer; ICIs: immune check point inhibitors. NGS: Next-generation sequencing.

NGS for liquid biopsies using large panels (comprehensive genomic profiling)
Possibility of using CGP panels with liquid biopsies at both initial diagnosis and tumor progression
Reflex testing using CGP panels with liquid biopsies
Novel bioinformatics for easier assessment and a shorter turnaround time for transmission of reports
Expanding indications for liquid biopsies in thoracic oncology
Detection of minimal residual disease in early stage NSCLC in daily practice
Integration of a liquid biopsy as a screening tool for detection of lung cancer
Develop liquid biopsy testing for predictive biomarkers of ICIs efficiency
Combined somatic and germline genomic alterations in thoracic oncology for better decision making for precision oncology (prediction of primary drug resistance; prediction of drug toxicity)
Adopt the In Vitro Diagnostics/Devices regulations for in-house liquid biopsies
Develop diagnostic tests with liquid biopsies as complimentary to testing with tissue biopsies for diagnosis
Harmonize NGS use with liquid biopsies avoiding research use only testing and leading to universal use of CE-IVD testing
Consider liquid biopsies as a pivotal tool for the concept of integrative pathology
Liquid biopsies (blood, pleural fluid and pericadial effusion, cerebrospinal fluid) combined with tissue biopsies for diagnosis, prognosis and predictive biomarker evaluation in thoracic oncology
Recognition of liquid biopsies as a pivotal element of artificial intelligence in thoracic oncology
Open a national expert center (s) for liquid biopsies
Expert centers will propose external quality assurance schemes
Expert centers will propose innovative technologies
Expert centers will develop training programs of excellence (educational classes in bioinformatics)

Toward using large panels for in-house liquid biopsies

Currently in most institutions comprehensive genomic profiling of cf-DNA of patients with advanced NSCLC is mainly requested by multidisciplinary tumor boards [42]. However, with the arrival of new targeted therapies for lung cancer, the potential use of the tumor mutational burden as a predictive biomarker of immune checkpoint inhibitors (ICIs), the development of new genomic signatures of interest such as the MSI, and the methylome, will certainly lead to a more systematic use of large panels (over 300 genes) when doing LBs in daily practice [42]. In this setting, we have to underline a number of issues: i) the acquisition of devices that allow analysis with large panels with cf-DNA, ii) the evaluation of the increased costs of using these large panels as well as the optimization of the bioinformatic pipelines to deliver the data in a turnaround time compatible with therapeutic decision making, iii) the assessment of the impact on the workload of staff members, iv) the necessity to upgrade knowledge of the physicians, technicians and pathologists to better interpret the increasing complexity of the molecular results.

New indications of liquid biopsies in thoracic oncology

Besides the detection of druggable genomic alterations in lung cancer patients and the detection of different mechanisms of resistance at tumor progression, other domains of interest will soon be developed in the daily practice of thoracic oncologists. First the detection of minimal residual disease (MRD), notably in the early stage of NSCLC, represents an opportunity to increase the development of LBs in daily practice, even if certain issues still need to be assessed (e.g, notably, the time points for blood sampling, the cutoff of cf-DNA needs to be defined, which test to use, the cost and reimbursement, the clinical validation for its usefulness) [43–45]. Second, due to the different programs of lung cancer screening and the development of different blood tests for diagnosis of lung cancer, LBs could be developed for better evaluation of pulmonary nodules in combination with low dose CT scans or to decide or not to perform low-dose computed tomography (LDCT) depending on the results of the blood tests [40,45]. When attempting to analyze very low concentrations of circulating tumor nucleic acid in early stage NSCLC a few issues are still being evaluated since it is pivotal to adopt highly sensitive molecular approaches [46]. Finally, due to the increase in the development of different ICIs, different LBs tests for predictive biomarkers of response to ICIs could be used in the near future [47].

Perspectives for new regulations for the use of in-house liquid biopsies: the in vitro diagnostics/devices regulation in Europe

The future in vitro diagnostics regulation (IVDR) needs in the near future to take into consideration the use of LBs [48]. This new regulation will probably have an impact on the possible use of RUO panels in-house. Moreover, the EU in vitro diagnostic device regulation (IVDR) aims at transparent risk- and purpose-based validation of diagnostic devices, traceability of results to uniquely identified devices, and post-market surveillance [49].

Develop center (s) of expertise for liquid biopsies at each national level

Thanks to the government authorities and also or not to some private partnerships, different initiatives could be develop in order to create at each national level one or several expert centers for liquid biopsies (Fig. 3). These centers of excellence will aim to disseminate different crucial information such as those related to the international guidelines

Fig. 3. Build center of expertise approach to drive supply and demand of liquid biopsies at the national level.

and recommendations, to new innovative devices and new panels, and will be able to propose on site trainings for external teams and to create network of stakholders working in the field of liquid biopsy.

Conclusion

As the demand for diagnostics for analysis of many solid cancers, notably for a number of predictive biomarkers in lung cancer, continue to grow, pathologists are constantly challenged by their clinical colleagues to meet their expectations both in terms of the comprehensiveness of testing and timeliness of results. However, questions as to the optimal approach to biomarker testing in daily practice remain. In this context, LBs nowadays provide opportunities to facilitate treatment decision making for advanced NSCLC and probably in the near future for early NSCLC [50]. However, some constraints can be present in this in-house setting, notably to master the pre-analytical phase, but also the cost efficiency and the setup of a new organization for care. Therefore, each institution has to build its own strategy to successful implement the use of LBs in daily practice for lung cancer genotyping [50]. LBs should become a regular tool in thoracic oncology, notably at initial diagnosis and at tumor progression, as a complementary approach to molecular testing with tissue biopsies. We strongly believe that LBs in thoracic oncology should be part of or be integrated into the expertise of the molecular pathologist [51]. It is essential that the different stakeholders adopt a common point of view to developing LBs wherein the objective is to not replace tissue biopsies, the only way to diagnose lung cancer [2,52]. However, different algorithms can be set up for using LB at the initial diagnosis (Fig. 2). The future in lung cancer could be systematic association at the same time of tissue biopsies and LBs at initial diagnosis and at tumor progression, but this practice is not yet indicated in the different guidelines and needs to be better evaluated to confirm its usefulness [28, 53–56].

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Declaration of competing interest

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